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**THE DETERMINATION OF MUSTARD AND THIODIGLYCOL IN  
MUSTARD HYDROLYSATE (U)**

by

P.A. D'Agostino and L.R. Provost

PCN No. 13E50

August 1984



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**ABSTRACT**

The mustard stored at the Defence Research Establishment Suffield was disposed of by hydrolysis during the 1970's. Samples of the liquid and sludge hydrolysate were analysed by gas chromatography with flame ionization and mass spectral detection for residual mustard and thiodiglycol, the major hydrolysis product of mustard. Trace quantities of mustard were found in two sludge hydrolysate samples. Thiodiglycol was found as a major component in both the sludge and liquid hydrolysate samples.

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### **ACKNOWLEDGEMENTS**

The authors wish to thank Dr. P.A. Lockwood's group for providing a purified mustard sample, Mr. W.N. Lawson and the Decontamination Unit for collecting the hydrolysate samples and Mr. J.P. Bitz for making the glass columns used for packed column gas chromatographic analysis. Thanks are also extended to Mr. B.G. Cameron for his advice during this study.

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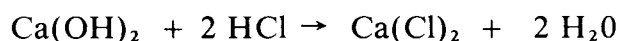
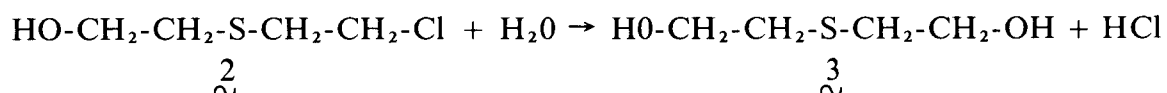
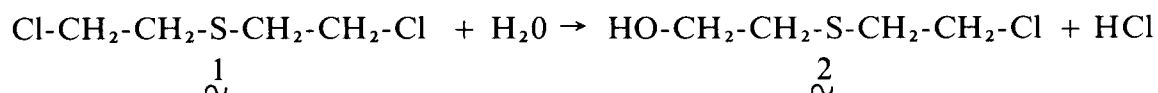
P.A. D'Agostino and L.R. Provost

**INTRODUCTION**

1. During World War II over 700 tons of the chemical warfare agent mustard were shipped to the Defence Research Establishment Suffield (DRES) and stored in five lead-lined concrete vaults (1). In the early 1970's it was decided that this stockpile of mustard would be destroyed by batch hydrolysis. Batch hydrolysis using 1000 gallons of mustard, 5000 pounds of lime ( $\text{Ca}(\text{OH})_2$ ) and 2500 gallons of water was carried out according to a method developed at DRES (2,3).

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2. The principle reactions involved in the hydrolysis of mustard (H) are shown in the equations below (4). Conversion of mustard  $\underset{\sim}{1}$  through hemisulfur mustard  $\underset{\sim}{2}$  to thiodiglycol  $\underset{\sim}{3}$  was essentially complete provided the ratio of water to mustard was large, the temperature was elevated to 100°C and the pH was maintained above 7 (3).



3. Following batch hydrolysis the mustard hydrolysate was transferred from the reaction vessel into one of five empty storage vaults numbered 6, 7, 8, 9 and 10. After a cooling and settling period the hydrolysate separated into two layers. The upper or liquid layer was very fluid and ranged from clear to pale yellow in colour. The lower or sludge layer was paste-like and yellow-brown in colour. Samples of the liquid and sludge layers from each of the five vaults containing the mustard hydrolysate were used for analysis.

4. The objective of this study was to develop an analytical method for the determination of thiodiglycol and mustard in the liquid and sludge layers of the mustard hydrolysate. The chloroform extracts of both the liquid and sludge layers of the storage vaults were initially screened by packed column gas chromatography (GC) with flame ionization detection (FID). Determination of residual mustard in the chloroform extracts was performed by packed column gas chromatography-mass spectrometry (GC-MS) in the selected-ion-monitoring (SIM) mode. Thiodiglycol was determined in both the liquid layer and the water extract of the sludge layer by packed column GC-FID and confirmed by packed column GC-MS.

## MATERIALS AND METHODS

### Materials

5. The chloroform and water were "HPLC grade" purchased from Fisher Scientific Company (Edmonton, Alberta). Thiodiglycol was purchased from Pierce Chemical Company (Rockford, Illinois). Purified mustard was provided by the Organic Chemistry Laboratory at DRES.

### **Mustard Hydrolysate Samples**

6. The mustard hydrolysate was stored in vaults 6, 7, 8, 9 and 10 at DRES. Samples of the liquid and sludge hydrolysate from each vault were supplied in polyethylene bottles by the DRES Decontamination Unit. The samples were coded east (E) or west (W) to indicate their position in the storage vaults. A liquid and sludge hydrolysate sample from each vault was analysed for the presence of mustard and thiodiglycol.

### **Liquid Hydrolysate Sample Preparation**

7. Seventy mL of each liquid hydrolysate sample were filtered using a 13 mm Swinny stainless steel filtering unit with a 1.0  $\mu\text{m}$  Duralon filter (Millipore Corp., Bedford, MA) and a 50 mL teflon Leur lock Hamilton syringe (Chromatographic Specialties Ltd., Brockville, Ontario). A 5 mL aliquot of the filtered liquid was used for the determination of thiodiglycol.

8. A 50 mL aliquot of the filtered liquid was extracted with chloroform (1  $\times$  10 mL and 2  $\times$  5 mL) using a glass separatory funnel with a teflon stopcock. The chloroform extract of the liquid hydrolysate was concentrated to dryness under a gentle stream of nitrogen. The residue was dissolved in 1 mL of chloroform and used for the determination of mustard.

### **Sludge Hydrolysate Sample Preparation**

9. The water extract of each sludge hydrolysate sample was used for thiodiglycol analysis. One gram of sludge hydrolysate from each vault was shaken vigorously for 3 minutes with 3 mL of water in a 10 mL polycarbonate Oak Ridge centrifuge tube (model 3118-0010, Fisher Scientific Co., Edmonton, Alberta). Samples were centrifuged at 3500  $\times$  g for 15 minutes and 1 mL of the supernatant was removed for the determination of thiodiglycol.

10. The chloroform extract of each sludge hydrolysate sample was used for mustard analysis. One gram of sludge hydrolysate was placed in an 8 mL vial having a teflon-lined screw cap. Three mL of chloroform were added and the vial was shaken vigorously for 3 minutes. The contents were allowed to settle for one hour. A 1.5 mL aliquot of the chloroform layer (lower) was removed and filtered through a 0.5  $\mu\text{m}$  teflon filter prior to mustard determination.



11. Water and chloroform blanks were treated in the same manner as the liquid and sludge hydrolysate samples. The blanks were examined prior to the vault samples to assess contamination during sampling handling. Hydrolysate samples and blanks were stored in vials having teflon-lined screw caps at 2°C to minimize contamination and evaporation.

### **Instrumental Analysis**

12. A Varian 3700 (Varian Associates, Georgetown, Ontario) gas chromatograph was used for packed column GC-FID and GC-MS analyses. The glass columns were custom made by the DRES glassblowing shop and were prepared and packed according to the method of Leibrand and Dunham (5). Packed column GC-FID operating conditions are listed in Table I.

13. Packed column GC-MS analyses were performed in both the scanning and selected-ion-monitoring (SIM) mode using a VG Micromass 70/70H double-focusing mass spectrometer (VG Analytical, Wythenshawe, UK). Operating conditions for packed column GC-MS are presented in Table II.

## **RESULTS AND DISCUSSION**

14. The analytical scheme used for the determination of mustard and thiodiglycol in the DRES mustard hydrolysate is illustrated in Figure 1. Thiodiglycol was determined in both the liquid layer and the water extract of the sludge layer by packed column GC-FID and confirmed by GC-MS in the scanning mode. Chloroform extracts of the liquid and sludge samples were used for the determination of mustard as suggested in the NATO Sampling guidelines (6). Mustard was quantitated by packed column GC-MS in the selected-ion-monitoring (SIM) mode.

15. Tenax packing was used for the GC analysis of thiodiglycol since this material is suitable for use with aqueous samples. Two packed GC columns (OV-101 and OV-17) and one fused silica capillary column (DB-5) were evaluated for the analysis of mustard in hydrolysate samples. Unless the chloroform extracts were diluted severe overloading of the capillary column occurred. Consequently capillary column GC analysis was not practical. The OV-101 packed column stationary phase provided the best peak shape and was used for the analysis of mustard.

16. A series of thiodiglycol standards in water and mustard in chloroform were prepared to evaluate the detection limit and linearity of the packed column GC-FID method. Thiodiglycol (0.0016 to 10 mg/mL) and mustard (0.004 to 2.6 mg/mL) were detected linearly over the ranges tested with correlation coefficients of 0.994 and 0.9997 respectively. The FID response curves are illustrated in Figure 2.

17. Packed column GC-FID detection limits of 1 ng for mustard and 2 ng for thiodiglycol, based on a signal to noise ratio of 3:1, correlated well with a previous study (7). A packed column GC-MS (SIM) detection limit of 0.5 ng was determined similarly for mustard. This detection limit translates into a sample detection limit of 0.8  $\mu\text{g H}$  per gram of sludge and 0.01  $\mu\text{g H}$  per mL of liquid based on a 2  $\mu\text{L}$  injection of the chloroform extract. Thiodiglycol was present in all samples at levels well above the packed column GC-MS detection limit.

18. Quantitation of thiodiglycol in the liquid and sludge hydrolysate was done by external calibration using FID peak heights. This was accomplished by substitution of the sample peak height values into the equation generated by the thiodiglycol response data illustrated in Figure 2. Thiodiglycol was found in the 6.2 to 13.9 mg per gram range in the water extract of sludge hydrolysate and 2.2 to 10.3 mg per mL range in the liquid hydrolysate as summarized in Table III. The GC-FID chromatograms of a sludge extract and a liquid layer are illustrated in Figure 3. Thiodiglycol and several other hydrolysate components were identified by packed column GC-MS. The electron impact mass spectrum of thiodiglycol found in vault 7 is presented in Appendix 1.

19. Preliminary study indicated that another hydrolysate component eluted with a retention time similar to that of mustard. As a result, quantitation could not be performed by packed column GC-FID. Packed column GC-MS in the selected-ion-monitoring mode, a technique that is highly selective and sensitive, was used to overcome this problem. The ions monitored were  $m/z$  109, 111, 158 and 160 (based on the electron-impact mass spectrum for mustard illustrated in Appendix 1). Triplicate analyses of the sample and sample plus mustard standard (20 – 25 ng) were performed for each chloroform extract of the liquid and sludge hydrolysate.

20. Trace levels of mustard were detected in the sludge hydrolysate of vaults 6 and 8. Figure 4 illustrates the selected-ion-monitoring traces for the co-injection of the vault 8 sludge sample plus mustard standard and the sludge sample alone. Confirmation was

based on both correct GC retention time and m/z 109 and 111 ion ratios. Quantitation, using the method of standard addition, was based on the m/z 109 and 111 ions since interference was present for m/z 158 and 160. Mustard was determined at 2.9 and 4.2  $\mu\text{g/g}$  of sludge (refer to Table III) in vaults 6 and 8 respectively. No mustard was detected in the chloroform extracts of the liquid hydrolysate samples using this method. This was expected since mustard hydrolyses rapidly in aqueous solutions.

21. Chloroform and water blanks were analysed by packed column GC-FID prior to the samples. No interferences were observed in the blanks.

### CONCLUSIONS

22. The hydrolysis of the mustard stored at DRES was essentially complete since only trace levels of mustard remained. Thiodiglycol, the principle hydrolysis product of mustard, was found to be a major component in the samples studied. The identity of other components in the hydrolysate will be discussed in a future publication.

1 Mustard: 1,1'-thiobis[2-chloroethane], Registry No. [505-60-2]

2 Hemisulfur mustard: 2-(2-chloroethyl)thioethanol, Registry No. [693-30-1]

3 Thiodiglycol: 2,2'-thiodiethanol, Registry No. [111-48-8]

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6. NATO Handbook for the Sampling and Identification of Chemical Warfare Agents, Volume 1 (Sampling and Identification Procedures and Techniques). AEP-10, 1979.

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**TABLE I**  
**PACKED COLUMN GC-FID CONDITIONS**

	<b>THIODIGLYCOL DETERMINATION</b>	<b>MUSTARD DETERMINATION</b>
GC COLUMN:	1.22 m × 1.5 mm i.d. Tenax GC, 60/80 mesh (Alltech Assoc., Arlington, IL)	1.22 × 1.5 m i.d. 5% OV 101 on 80/100 mesh Chromosorb W (Chromatographic Specialties Ltd., Brockville, Ont)
TEMPERATURE PROGRAM:	150° for 1 min, then 10°/min to 250°C and held for 5 min	50° for 2 min, then 5°/min to 250°C and held for 10 min
INJECTION TEMPERATURE:	250°C	250°C
CARRIER GAS:	High purity helium <sup>a,c</sup> at 20 mL/min	High purity helium <sup>a,c</sup> at 25 mL/min
FID GASES:	Zero air <sup>b,c</sup> at 300 mL/min, ultra high purity hydrogen <sup>b,c</sup> at 30 mL/min	
FID TEMPERATURE:	250°C	

a Helium is passed through Drierite, molecular sieve, dust and oxygen removal filters.

b Air and hydrogen are passed through Drierite, molecular sieve and dust filters.

c Gas supplier: Liquid Carbonic Canada Ltd. (Scarborough, Ontario).

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**TABLE II**  
**PACKED COLUMN GC-MS CONDITIONS**

<b>OPERATING PARAMETERS</b>	<b>THIODIGLYCOL DETERMINATION</b>	<b>MUSTARD DETERMINATION</b>
GC COLUMN:	1.22 m × 1.5 mm i.d. Tenax GC, 60/80	1.83 × 1.5 mm i.d. 5% OV 101 on 80/100 mesh Chromosorb W
GC-MS INTERFACE:	Jet Separator (230°C)	
IONIZATION MODE:	Electron Impact	
ELECTRON ENERGY:	70 eV	
EMISSION:	200 μA	
SOURCE TEMPERATURE:	190 – 200°C	
SOURCE PRESSURE:	ca. $2 \times 10^{-6}$ torr	
SCAN FUNCTION AND RATE:	350 to 20 amu, exponential down scan, 3 sec/decade	Selected-ion-monitoring of m/z 100 (PFK lock mass) 109, 111, 158 and 160 with 200 msec/ion dwell time
ACCELERATING VOLTAGE:	4 kV	Stepped from 4 kV downwards
RESOLUTION (10% VALLEY DEFINITION):	500	250 – 300

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**TABLE III**  
**THIODIGLYCOL (TDG) AND MUSTARD (H) CONCENTRATIONS IN**  
**THE DRES MUSTARD HYDROLYSATE**

VAULT NUMBER (W:West; E:East)	LIQUID HYDROLYSATE <sup>a</sup> mg TDG/mL liquid <sup>b</sup>	SLUDGE HYDROLYSATE	
		mg TDG/g sludge <sup>b</sup>	μg H/g sludge <sup>b</sup>
6 W	4.7 ± 0.1	11.8 ± 0.9	2.9 ± 0.5
7 W	4.4 ± 0.2	7.7 ± 0.5	ND
8 W	2.2 ± 0.1	6.2 ± 0.3	ND
9 W	10.3 ± 0.5	13.9 ± 0.3	4.2 ± 0.8
10 W	6.1 ± 0.4		
10 E <sup>c</sup>		12.0 ± 0.5	ND

a Mustard was not detected in the liquid hydrolysate samples. Levels are below 0.01 μg/mL liquid based on the selected-ion-monitoring detection limit of 500 pg for H (S/N = 3:1).

b Concentrations given as mean ± standard deviation (n = 3).

c Since no sludge sample from vault 10 W was available, one from 10 E was used for sludge analysis.

ND < 0.8 μg H/g sludge based on the selected-ion-monitoring detection limit of 500 pg for H (S/N = 3:1).

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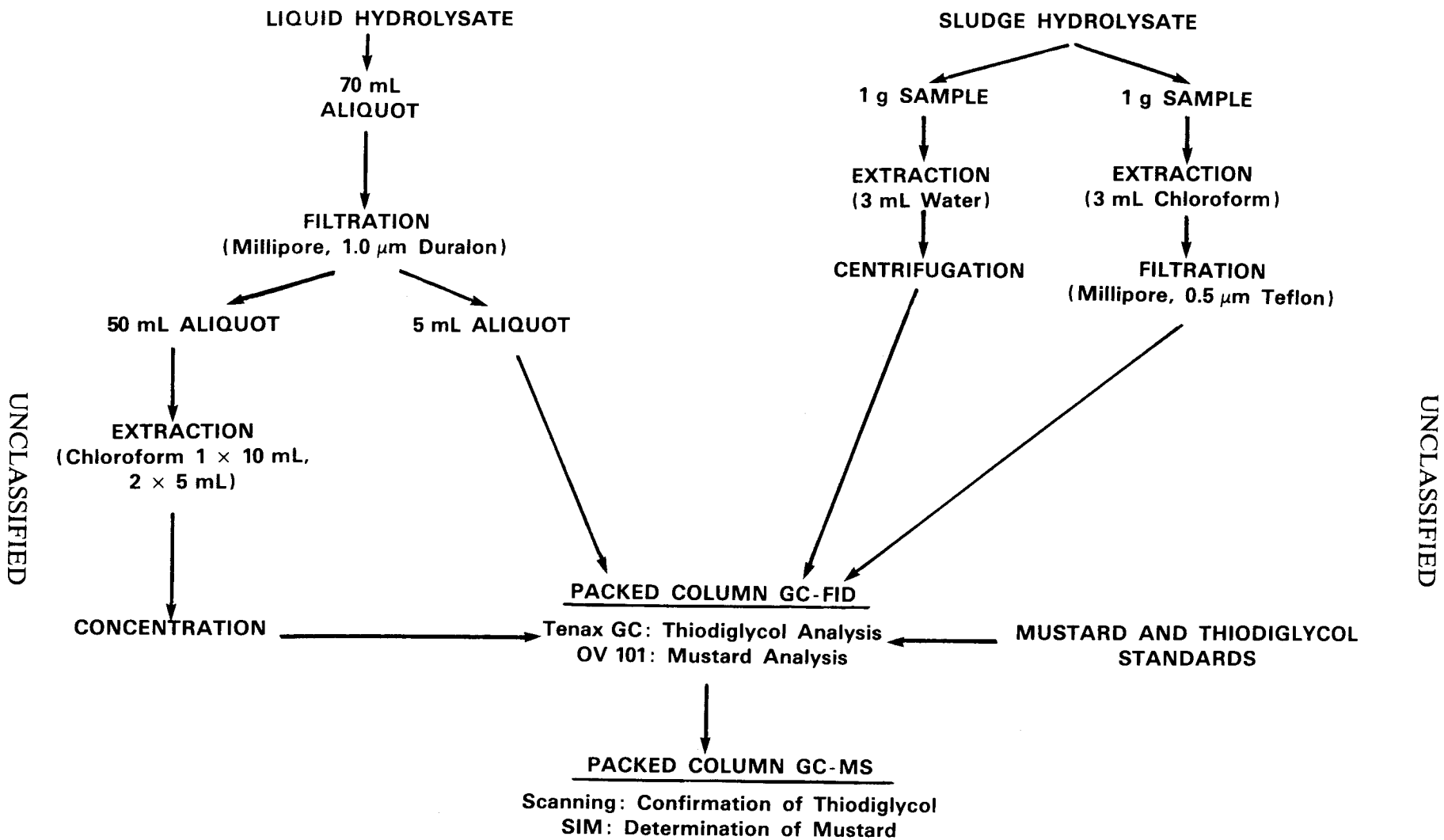


Figure 1

ANALYTICAL SCHEME FOR THE DETERMINATION OF MUSTARD AND THIODIGLYCOL IN THE MUSTARD HYDROLYSATE

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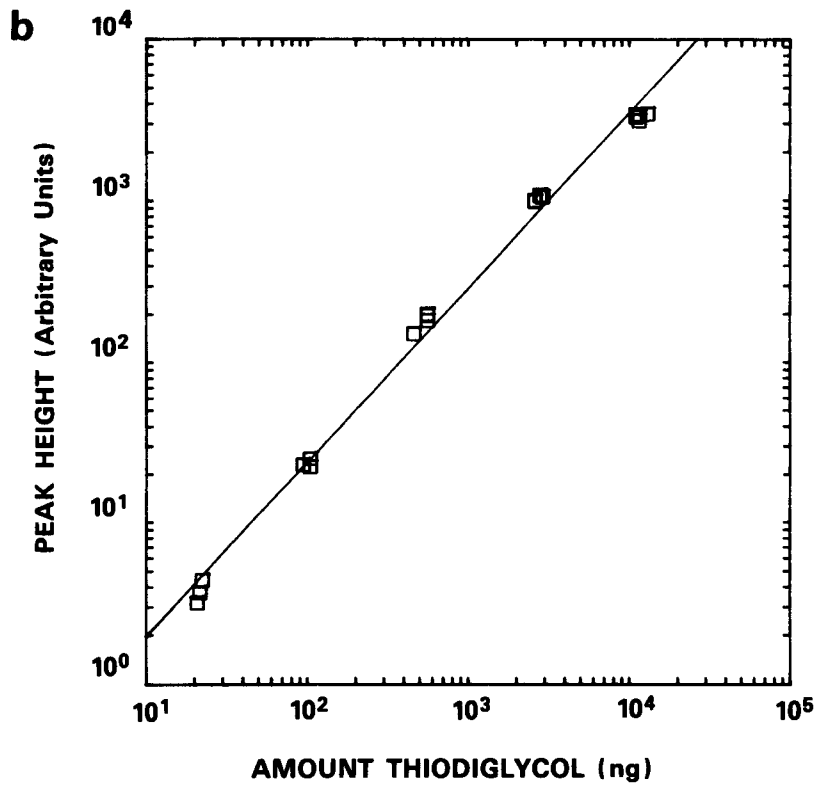
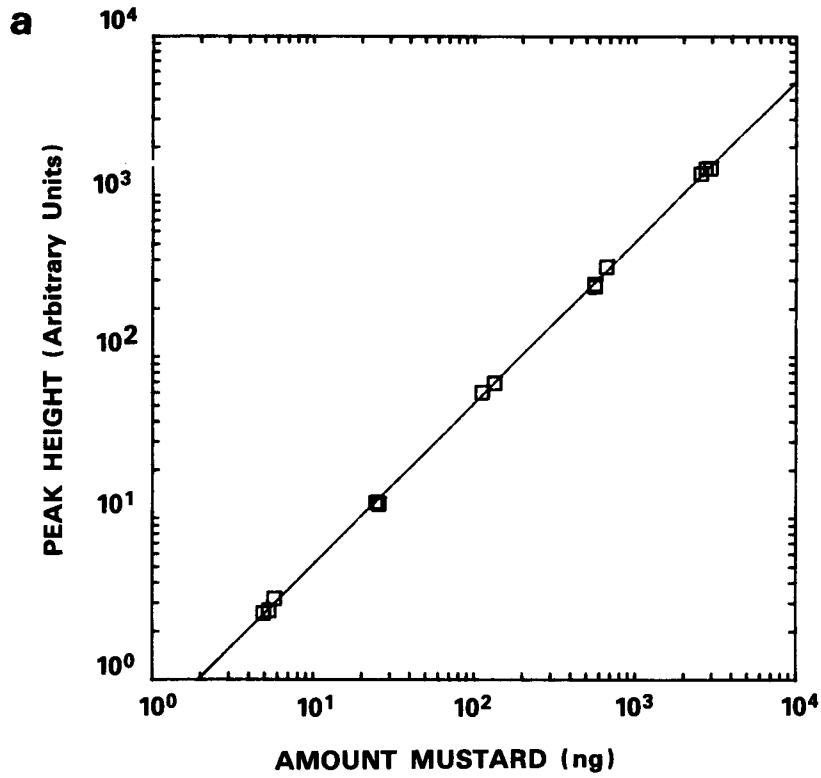


Figure 2

PACKED COLUMN GC-FID CALIBRATION CURVES FOR  
a) MUSTARD AND b) THIODIGLYCOL. NOTE THE LOG SCALES

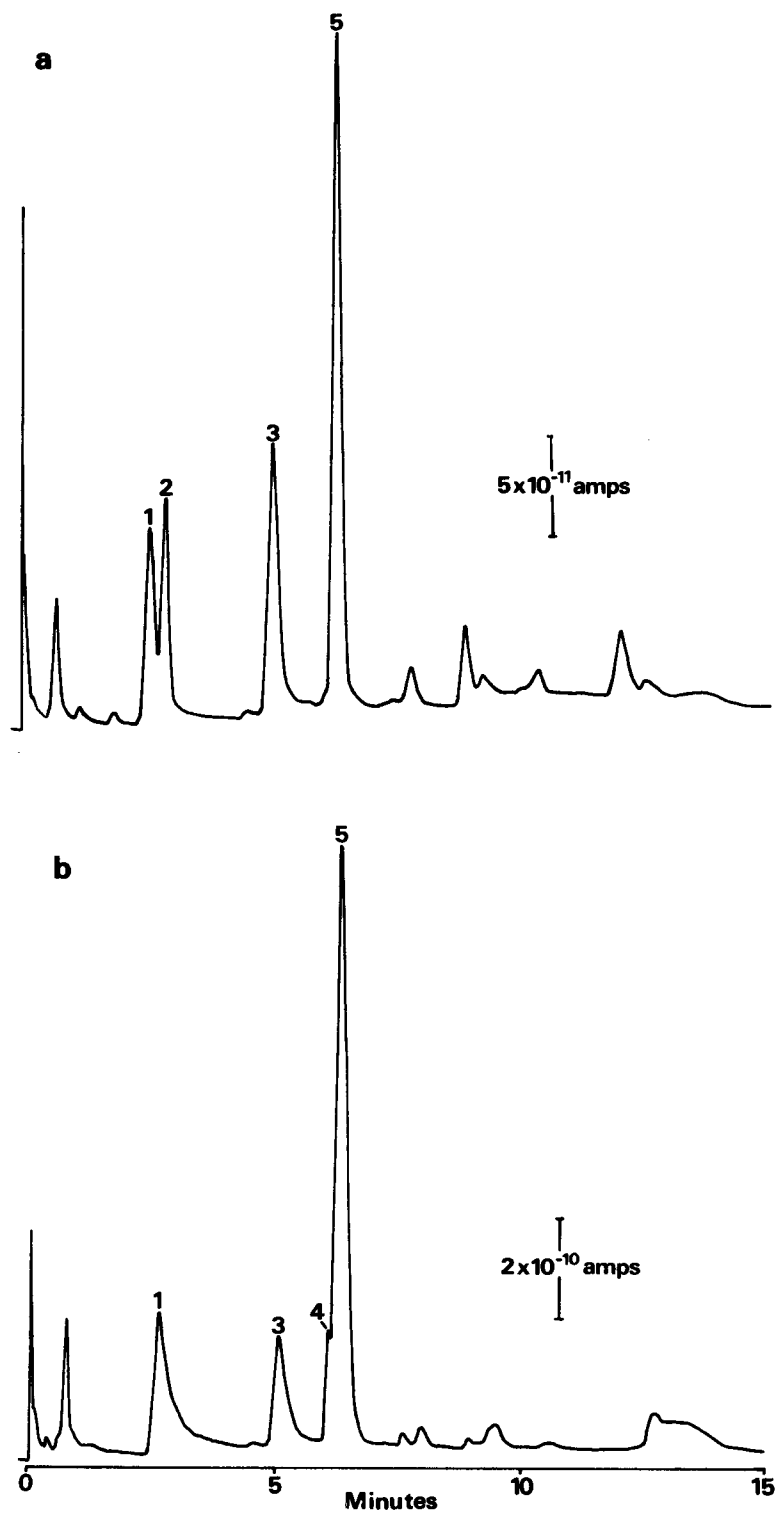


Figure 3

PACKED COLUMN GC-FID CHROMATOGRAMS OF a) WATER EXTRACT OF THE EQUIVALENT OF 380  $\mu\text{g}$  OF VAULT 7 SLUDGE HYDROLYSATE AND b) 1.1  $\mu\text{L}$  OF VAULT 9 LIQUID HYDROLYSATE. COMPOUNDS IDENTIFIED: 1,4-THIOXANE (1), AN UNKNOWN (2), 1,4-DITHIANE (3), HEMISULFUR MUSTARD (4) AND THIODIGLYCOL (5).

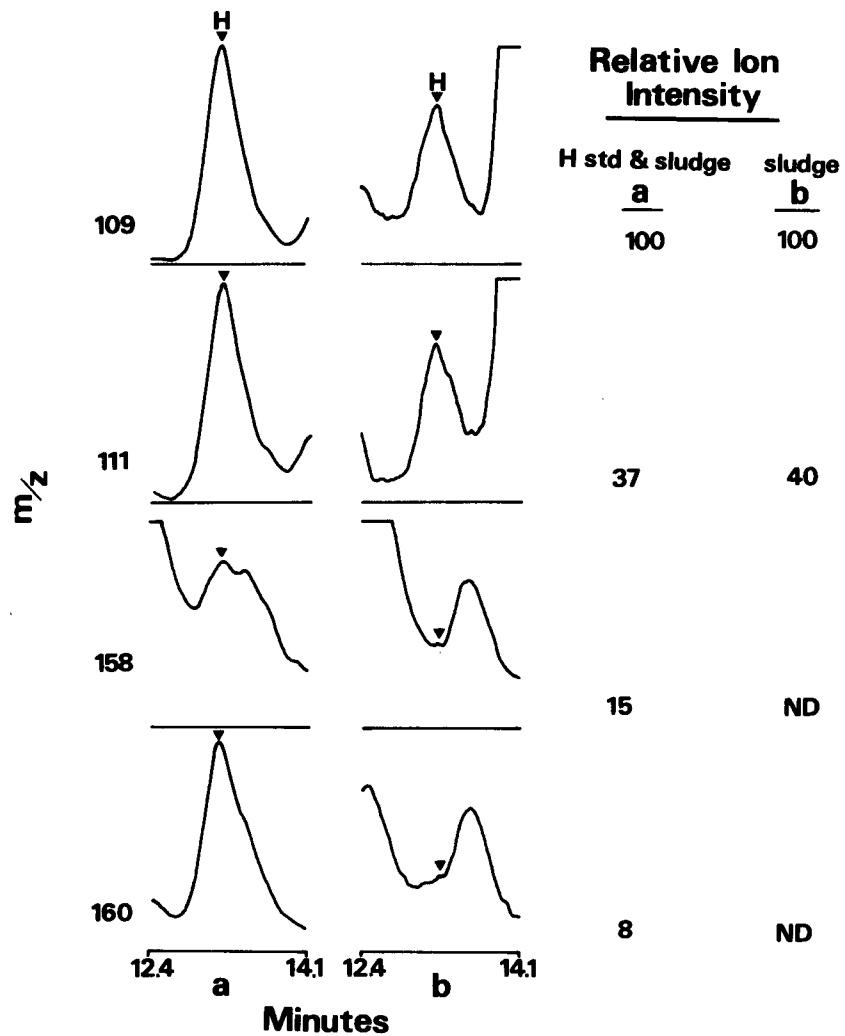
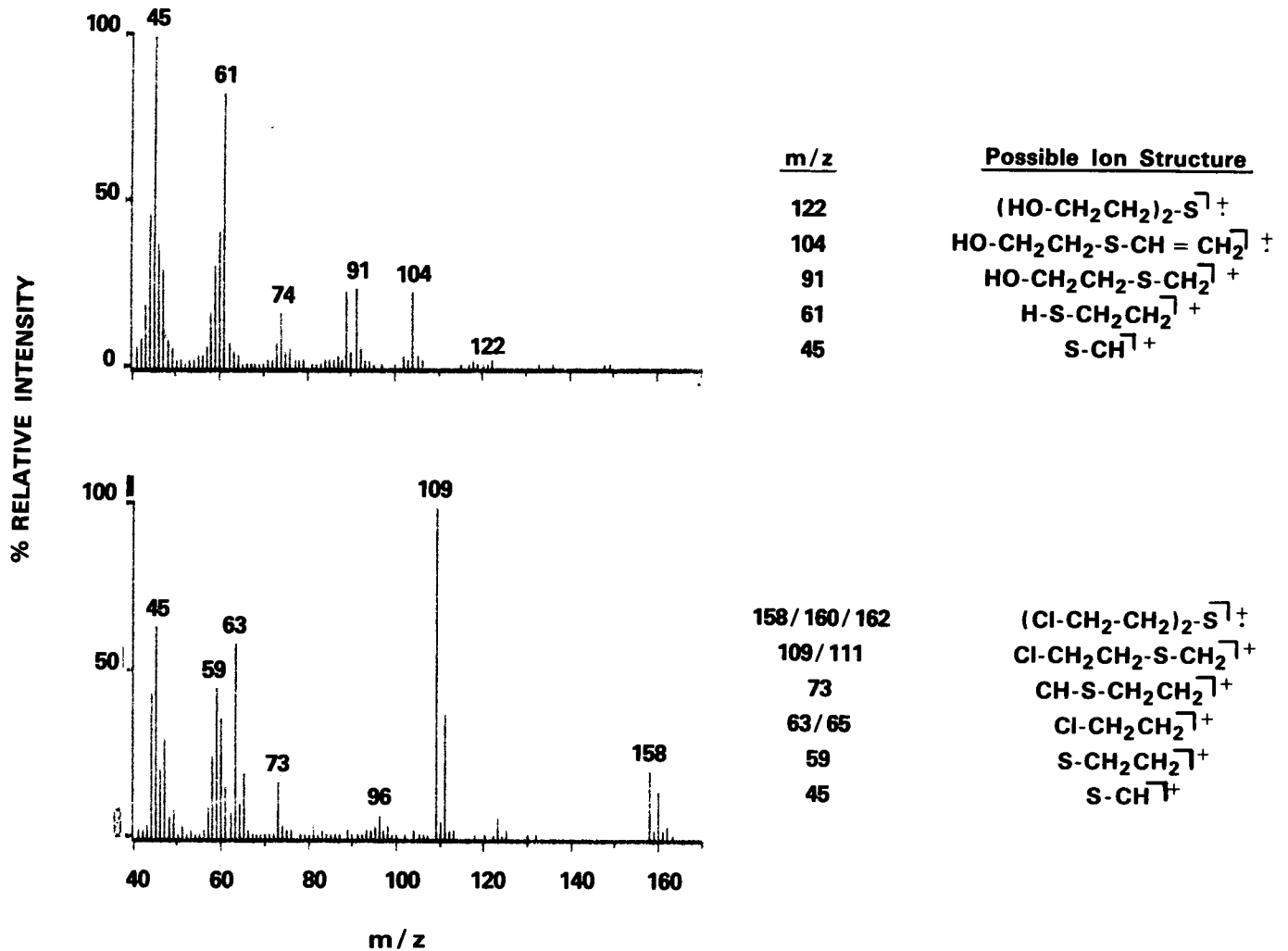


Figure 4

DETERMINATION OF MUSTARD (H) IN VAULT 8 SLUDGE HYDROLYSATE BY PACKED COLUMN GC-MS WITH SELECTED-ION-MONITORING OF  $m/z$  109, 111, 158 AND 160. a) TRACES OBTAINED BY THE CO-INJECTION OF 25 ng H AND THE EQUIVALENT OF 440  $\mu\text{g}$  OF SLUDGE HYDROLYSATE b) TRACES OBTAINED BY THE EQUIVALENT OF 850  $\mu\text{g}$  OF SLUDGE HYDROLYSATE (ND: NOT DETECTED).



## Appendix I

**ELECTRON-IMPACT MASS SPECTRUM OF a) THIODIGLYCOL IDENTIFIED IN VAULT 7 SLUDGE HYDROLYSATE AND b) MUSTARD STANDARD USING PACKED COLUMN GC-MS.**

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## KEY WORDS

Gas Chromatography  
 Mass Spectroscopy  
 Solvent Extraction  
 Chemical Agent Detection  
 Military Chemical Agent  
 Mustard Agents  
 Thiodiglycol  
 Mustard Hydrolysate  
 (560-60-2)  
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